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基于线粒体 12S rRNA 和 16S rRNA 基因序列
的鹭科(Aves:Ardeidae)鸟类系统发生关系

Phylogenetic relationships among herons inferred from
mitochondrial 12S rRNA and 16S rRNA gene sequences

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摘要

本实验利用线粒体 rRNA 基因序列对我国分布的鹭科鸟类的系统发生关系做了初步研究。

鹭类的 12S rRNA 基因片段共有核苷酸变异位点 82 个, 简约信息位点 53 个。在已获得 12S rRNA 基因片段的 14 种鹭科鸟类中, 黄嘴白鹭 (*Egretta eulophotes*) 和黄苇鹀 (*Ixobrychus sinensis*) 的遗传距离最大, 为 0.11893。鹭亚科 (*Ardeinae*) 中, 遗传距离最大的是黄嘴白鹭 (*Egretta eulophotes*) 和大白鹭 (*Egretta alba*), 为 0.10846。鹀亚科 (*Botaurinae*) 中, 黑鹀 (*Dupetor flavicollis*) 和黄苇鹀 (*Ixobrychus sinensis*) 遗传距离最大, 为 0.09468。对夜鹭 (*Nycticorax nycticorax*)、白鹭 (*Egretta garzetta*) 的不同个体分别测序, 没有发现个体之间有碱基差异。

获得了 13 种鹭类的 16S rRNA 基因序列。其中鹭类 16S rRNA 基因核苷酸变异位点有 89 个, 简约信息位点 53 个。在已获得 16S rRNA 基因片段的 13 种鹭科鸟类中, 岩鹭 (*Egretta sacra*) 和黑鹀 (*Dupetor flavicollis*) 的遗传距离最大, 为 0.12887。鹭亚科中, 夜鹭 (*Nycticorax nycticorax*) 和大白鹭 (*Egretta albus*) 的遗传距离最大, 为 0.10808, 鹀亚科中, 海南鹀 (*Gorsachius magnificus*) 和大麻鹀 (*Botaurus stellaris*) 的遗传距离最大, 为 0.10396。白鹭 (*Egretta garzetta*) 和暗色型小白鹭 (black *Egretta garzetta*) 在碱基组成上完全相同。

12S rRNA 和 16S rRNA 基因碱基替换中转换和颠换距离与碱基差异的关系说明, 转换和颠换距离随着总的序列的变化的增加都呈线性增长, 而且转换积累的速率高于颠换, 转换和颠换都没有达到饱和。

利用 12S rRNA 基因构建系统进化树的结果支持将大白鹭和中白鹭分出白鹭属 (*Egretta*) 的观点, 但是 16S rRNA 基因构建的系统进化树则只支持将大白鹭独立出来, 而中白鹭仍然在白鹭属。同时后者也支持现有的对鹀亚科的分类, 即将鹀亚科分为夜鹀属 (*Gorsachius*)、苇鹀属 (*Ixobrychus*) 和麻鹀属 (*Botaurus*)。通过对白鹭和暗色型小白鹭的线粒体 rRNA 基因序

列比较证明暗色型小白鹭是白鹭，可能发生了羽色突变。

本文对外群的选择做了初步的探讨。外群的选择与该种与内群的分类地位上的远近没有直接关系，而是与基因之间的遗传距离相关；外群与内群的最小遗传距离不能低于内群之间的最大遗传距离。并且通过分析发现一般的研究者选用的外群与内群之间的碱基差异百分比都在 14% 以上。

同时，本文应用三种 DNA 提取方法(蛋白酶 K 法，Chelex 100 法，硅颗粒法)，从馆藏湿地鸟类陈旧标本的羽毛、皮肤、骨骼组织中提取基因组 DNA，经 PCR 扩增后对线粒体 12SrDNA 部分片段进行测序和 Genbank 比对，以探讨鸟类陈旧标本 DNA 的最佳提取方法和提取组织。结果表明，利用鸟类陈旧标本能够提取到基因组 DNA。蛋白酶 K 法和硅颗粒法能够从白鹭 (*Egretta garzetta*) 标本的骨骼组织中提取到 DNA 并获得长度 425bp 的线粒体 12SrDNA 序列 (Genbank 接收号 AY766387)，蛋白酶 K 法还能够从白鹳 (*Ciconia ciconia*) 标本的羽毛中获得长度 263bp 的线粒体 12SrDNA 序列 (Genbank 接收号 AY766388)，Chelex 100 法不能从鸟类标本中提取到 DNA。蛋白酶 K 法的 DNA 提取量较大，但是步骤繁琐而导致 DNA 被污染的几率增大，因此，实验过程中要注意防止污染，且提取骨组织 DNA 要经过纯化。硅颗粒法的步骤简单，污染几率降低，提取骨骼组织 DNA 不需经过纯化，但是其 DNA 提取量较少，不能用于羽毛 DNA 的提取。因此，硅颗粒法是从鸟类陈旧标本骨骼组织提取 DNA 的理想方法，蛋白酶 K 法可用于鸟类陈旧标本羽毛的 DNA 提取。

关键词：线粒体 rDNA；鹭科；系统进化

Abstract

The phylogenetic relationships among species of Ardeidae which distribute in China were assessed by mitochondrial rRNA gene sequences in this study.

The portions of 12S rRNA gene include 82 variable sites and 53 parsimony-informative nucleotide sites. Sequences of 12S rRNA genes of 14 species of Ardeidae are obtained, in which the genetic distance between *Egretta eulophotes* and *Ixobrychus sinensis* is the most prominent. It reaches 0.11893. The genetic distances between *Egretta eulophotes* and *Egretta alba* in Ardeinae, *Dupetor flavicollis* and *Ixobrychus sinensis* in Botaurinae are the most prominent. And the former is 0.10846, the later is 0.09468. 12S rRNA gene in different individuals of *Nycticorax nycticorax* and *Egretta garzetta* are sequenced, no difference in nucleotide bases is observed.

The portions of 16S rRNA gene include 89 variable sites and 53 parsimony-informative nucleotide sites. Sequences of 16S rRNA genes of 13 species of Ardeidae are obtained, in which the genetic distance between *Egretta sacra* and *Dupetor flavicollis* is the most prominent. It reaches 0.12887. The genetic distances between *Nycticorax nycticorax* and *Egretta alba* in Ardeinae, *Gorsachius magnificus* and *Botaurus stellaris* in Botaurinae are the most prominent. And the former is 0.10808, the later is 0.10396. No difference which is between *Egretta garzetta* and black *Egretta garzetta* of nucleotide bases in 12S rRNA and 16S rRNA genes is observed.

The relationships between the sequence divergences of transition and transversions and the total percentage of sequence divergences

of 12S rRNA gene and 16S rRNA gene show that the sequence divergences of transition and transversions go up with the increasing of the total percentage of sequence divergences in linearity. And the speed of accumulation of transition is faster than transversion, both transition and transversion do not saturate.

The phylogenetic tree of 12S rRNA gene shows that *Egretta alba* and *Egretta intermedia* should be separated from *Egretta*. But the phylogenetic tree of 16S rRNA gene supports the point that *Egretta intermedia* belongs to *Egretta*, it also shows that *Egretta alba* should be separated from *Egretta*. And at the same time it illustrates that Botaurinae includes *Gorsachius*, *Ixobrychus* and *Botaurus*. The comparison results of sequences of mitochondrial rRNA gene show that mutations are arisen in feather-color deciding genes, and the black egret is not new specie.

The problems of the choice of outgroup are studied. The proper outgroups are not those that close to ingroups in morphologic class, genetic distance between outgroup and ingroup is a more important factor which should be considered carefully. The genetic distances between outgroup and ingroup are more than 14% in general.

To find the better methods for extracting genomic DNA from old specimens of wetland bird, three methods, Proteinase K method, Chelex R 100 method, and Silica-based method, were used to extract genomic DNA from the feather, skin and bone tissues of the specimens. The obtained target fragments of 12SrDNA in the mtDNA were sequenced after the PCR amplification of the DNA and then were compared with the Genbank data. The results show that the old specimens of wetland

bird could be used for DNA extraction. Proteinase K method and Silica-based method both can extract genomic DNA from the bone tissue of *Egretta garzetta* specimen and obtain 425bp target fragments of 12SrDNA in the mtDNA (the receive number of Genbank, AY766387) , and Proteinase K also can extract genomic DNA from the feather of *Ciconia ciconia* specimen and obtain 263bp target fragments of 12SrDNA in the mtDNA (the receive number of Genbank, AY766388) . However, Chelex R 100 method cannot extract any genomic DNA from old specimens of wetland bird. More DNA could be extracted by using Proteinase K method. But Proteinase K method needs more steps in the DNA purification, which increase the probability of external DNA pollution. Therefore, particular attentions should be paid to avoid the external pollution when using Proteinase K method, and the DNA extracted from the bone tissue has better to be purified. The silica-based method is a simple one for DNA extraction, which not only can decrease the pollution probability but also the DNA extracted from the bone tissue needn' t to be purified, although the silica-based method extracted less DNA from the bone tissue than Proteinase K method and couldn' t extract DNA from the feather. It is suggested that silica-based method is used for DNA extraction from the bone tissue of bird specimens and the Proteinase K method is used for DNA extraction from the feather.

Key Words: mitochondrial rDNA; Ardeidae; phylogenetic

第一章 前言

1.1 鹭科分类问题探讨

1.1.1 鹭科鸟类的生物学特征

鹭科(Ardeidae)鸟类隶属于鸮形目(Ciconiiformes)。鹭科鸟类是大中型涉禽,嘴长而尖直,翅大而长,颈长且飞行时呈“S”型,脚和趾均细长,胫部部分裸露,中趾的爪上具梳状栉缘。体羽疏松,多为单色,通常由白、灰、紫、褐等色构成,有些种类具深色条纹。不少种类在头、背或前颈下部有丝状蓑羽,繁殖期尤为突出。雌雄同色。鹭类喜欢集群生活,栖息于江河、湖泊的滨岸及沼泽地带,白昼或黄昏活动,食性以鱼类为主,兼食蛙类、蛇类、昆虫、软体动物及小型啮齿类,常站在水边或浅水中,用嘴飞快的攫食。鹭类大多为迁徙种类,繁殖期多为群居,在树上、灌木丛或地面上用枝条筑造浅巢。每巢产卵3-6枚,卵呈蓝色、白色或皮黄色,无斑点。雌雄共同孵卵。雏鸟为晚成鸟^[1, 2]。

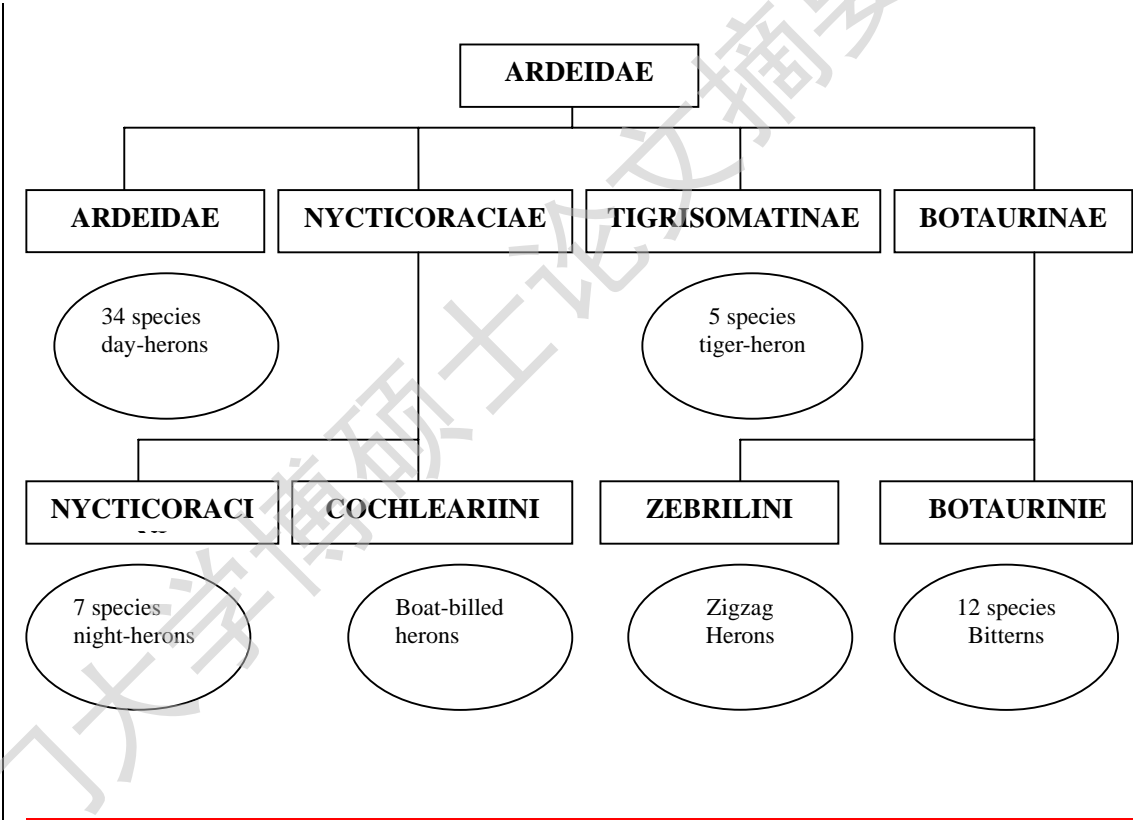
1.1.2 世界鹭科的分类

鹭科鸟类除了亚洲北部、北美洲北部、南极洲及一些海岛以外,广泛分布于世界各地^[1]。全球共有鹭科鸟类约60种。第一个完整而可靠的鹭科(Ardeidae)分类系统来自于W. J. Bock的著作。传统上,鹭科(Ardeidae)被划分成两个亚科:鴈亚科(Botaurinae)和鹭亚科(Ardeinae),从形态、结构以及行为特征考虑,两个亚科有明显的不同^[3]。Payne & Risley在对鹭科种类的33个骨骼性状进行数值分类研究并结合生态特征分析后,提出将鹭科分为4个亚科(图1)。Mayr & Cottrell认为全世界的鹭科有62种,按照Payne & Risley提出的分类系统,它们分别隶属于鹭亚科(Ardeinae)有6属36种;夜鹭亚科(Nycticoracinae)有3属18种;虎鹭亚科(Tigrisomatinae)有3属5种;麻鴈亚科(Botaurinae)有3属13种^[4]。Howard等认为世界鹭类共17个属118个亚种^[5]。Burt & Charles在他们

的书中收录了 65 种鹭类^[6]。

1. 1. 3 中国鹭科的分类

我国鹭类的分布主要集中在江南，东北、西北及青藏地区分布较少^[1]。中国鹭类有 20 种，4 亚种。按照传统的分类方法，将中国的鹭类分为两个亚科，鹭亚科 (Ardeinae) 和鴉亚科 (Botaurinae)，分别隶属于 9 个属 (图 2)。



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